Fig. 1b

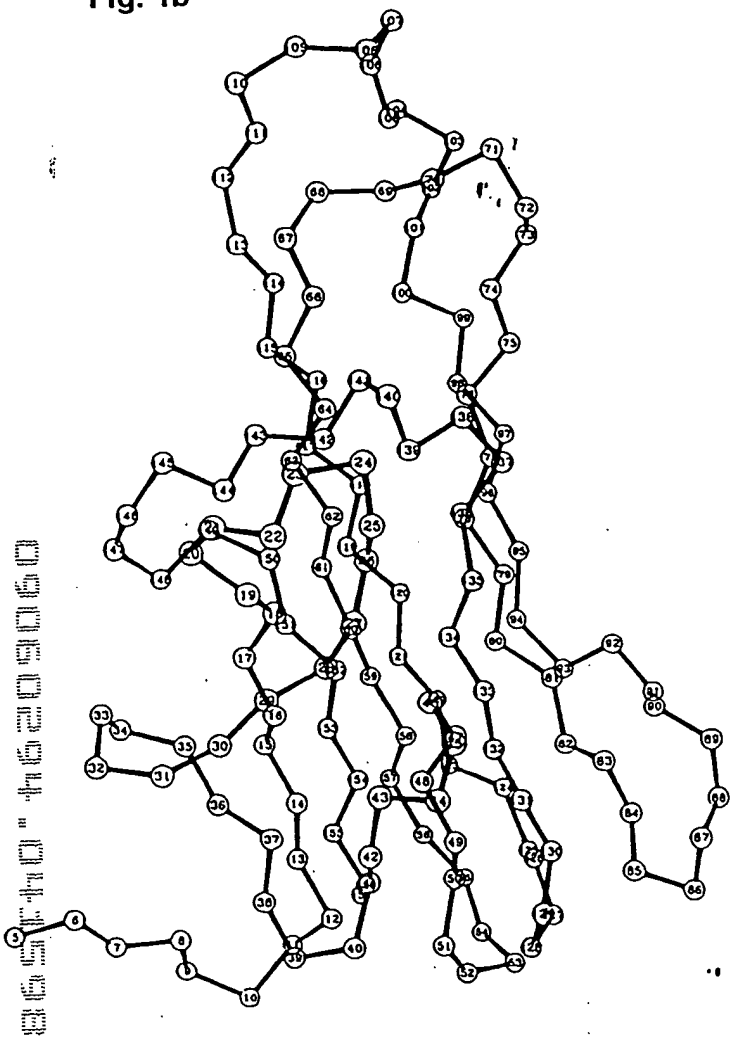


Fig. 1a

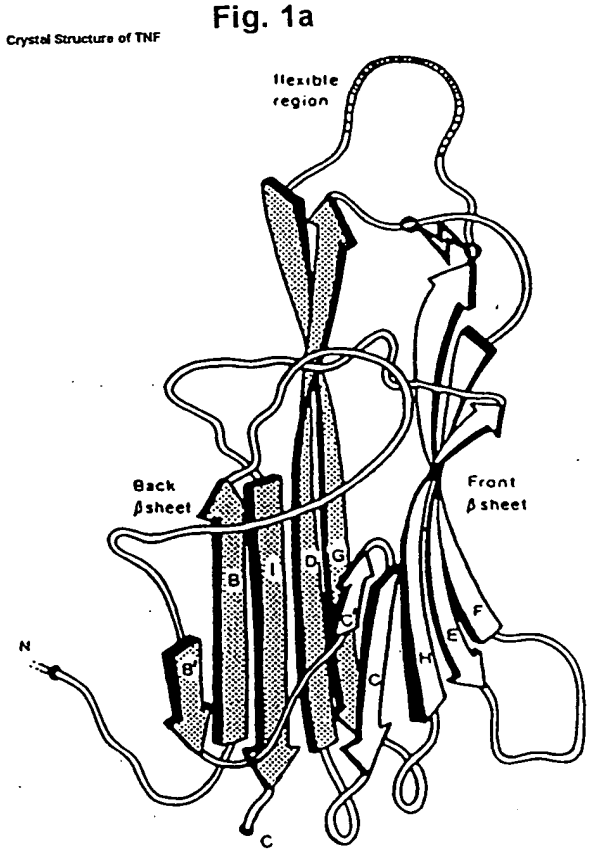


Fig. 1c

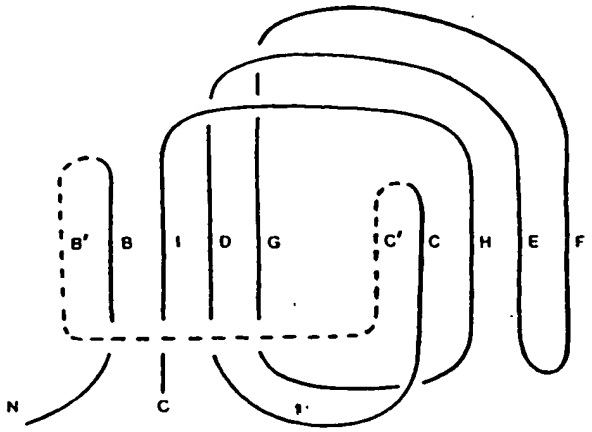


Fig. 2

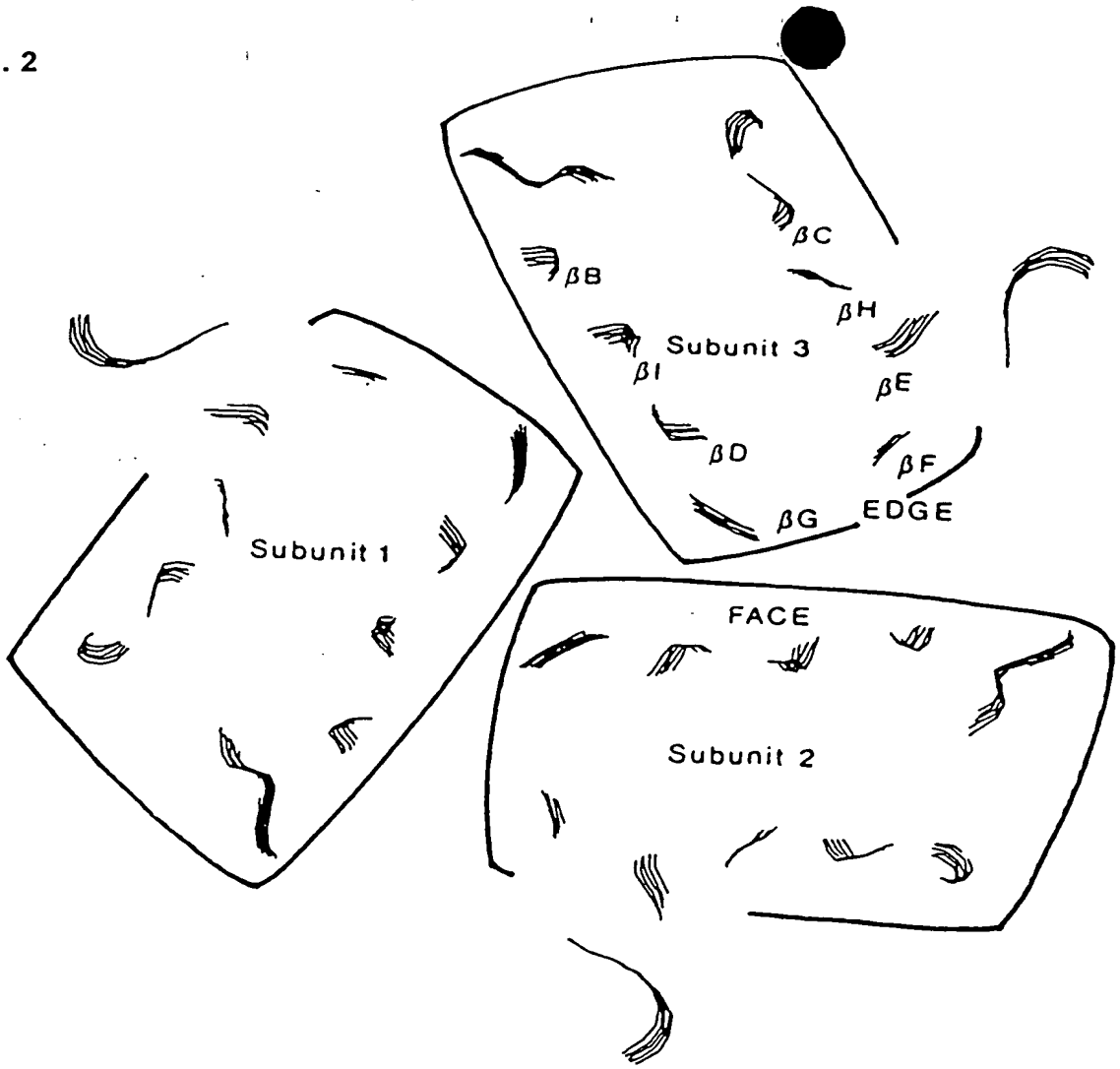


Fig. 3b

Amino acid sequence for human TNFα [8]

Accession code: Swissprot P01375

```
-76  MSTESMIRDV ELAEEALPKK TGGPQGSRRC LFLSLFSFLI VAGATTLFCL
-26  LHFGVIGPQR EEFPRDLSLI SPLAQA
  1  VRSSSRTPSD KPVAHVVANP QAEGQLQWLN RRANALLANG VELRDNQLVV
 51  PSEGLYLIYS QVLFKGQGCP STHVLLTHTI SRIAVSYQTK VNLLSAIKSP
101  CQRETPEGAE AKPWYEPIYL GGVFQLEKGD RLSAEINRPD YLDFAESGQV
151  YFGIIAL
```

Conflicting sequence F->S at position –14. Disulphide bond between Cys69–101. Signal anchor sequence –41 to –21 (underlined). Myristylation on Lys–58/–57.

Fig. 3a

```
   1 cacaccctga caagctgcca ggcaggttct cttcctctca catactgacc
  51 cacggctcca ccctctctcc cctggaaagg acaccatgag cactgaaagc
 101 atgatccggg acgtggagct ggccgaggag gcgctcccca agaagacagg
 151 ggggccccag ggctccaggc ggtgcttgtt cctcagcctc ttctccttcc
 201 tgatcgtggc aggcgccacc acgctcttct gcctgctgca ctttggagtg

 251 atcggccccc agagggaaga gtcccccagg gacctctctc taatcagccc
 301 tctggcccag gcagtcagat catcttctcg aaccccgagt gacaagcctg
 351 tagcccatgt tgtagcaaac cctcaagctg aggggcagct ccagtggctg
 401 aaccgccggg ccaatgccct cctggccaat ggcgtggagc tgagagataa
 451 ccagctggtg gtgccatcag agggcctgta cctcatctac tcccaggtcc

 501 tcttcaaggg ccaaggctgc ccctccaccc atgtgctcct cacccacacc
 551 atcagccgca tcgccgtctc ctaccagacc aaggtcaacc tcctctctgc
 601 catcaagagc ccctgccaga gggagacccc agagggggct gaggccaagc
 651 cctggtatga gcccatctat ctgggagggg tcttccagct ggagaagggt
 701 gaccgactca gcgctgagat caatcggccc gactatctcg actttgccga

 751 gtctgggcag gtctactttg ggatcattgc cctgtgagga ggacgaacat
 801 ccaaccttcc caaacgcctc ccctgcccca atccctttat taccccctcc
 851 ttcagacacc ctcaacctct tctggctcaa aaagagaatt gggggcttag
 901 ggtcggaacc caagcttaga actttaagca acaagaccac cacttcgaaa
 951 cctgggattc aggaatgtgt ggcctgcaca gtgaagtgct ggcaaccact

1001 aagaattcaa actggggcct ccagaactca ctggggccta cagctttgat
1051 ccctgacatc tggaatctgg agaccaggga gcctttggtt ctggccagaa
1101 tgctgcagga cttgagaaga cctcacctag aaattgacac aagtggacct
1151 taggccttcc tctctccaga tgtttccaga cttccttgag acacggagcc
1201 cagccctccc catggagcca gctccctcta tttatgtttg cacttgtgat

1251 tatttattat ttatttatta tttatttatt tacagatgaa tgtatttatt
1301 tgggagaccg gggtatcctg ggggacccaa tgtaggagct gccttggctc
1351 agacatgttt tccgtgaaaa cggaggctga acaataggct gttcccatgt
1401 agccccctgg cctctgtgcc ttcttttgat tatgtttttt aaaatattat
1451 ctgattaagt tgtctaaaca atgctgattt ggtgaccaac tgtcactcat

1501 tgctgaggcc tctgctcccc agggagttgt gtctgtaatc ggcctactat
1551 tcagtggcga gaaataaagg ttgcttagga aagaa
```

Fig. 4a

Location of inserted epitopes

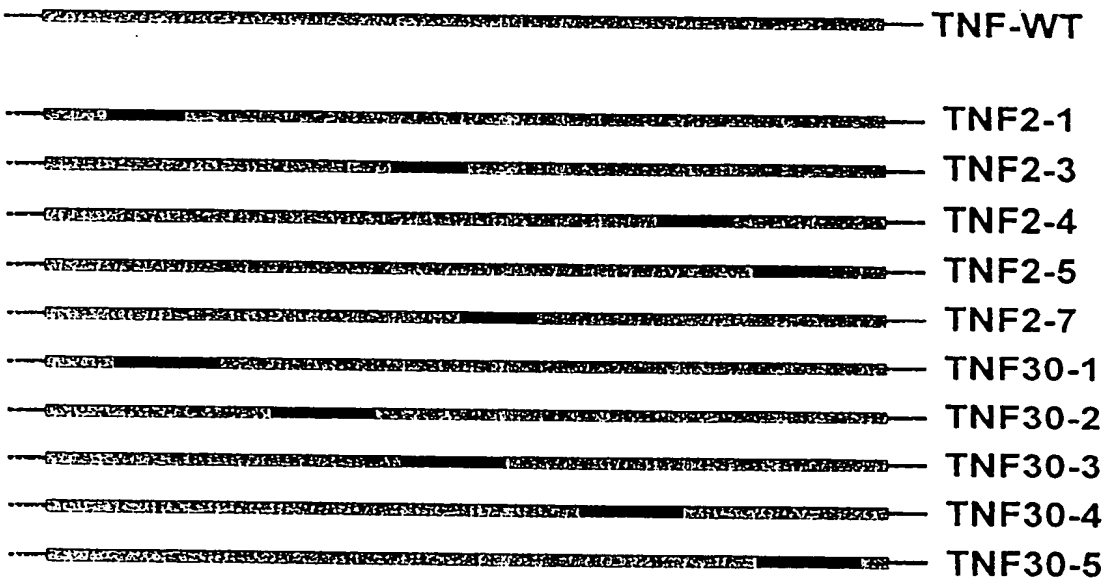
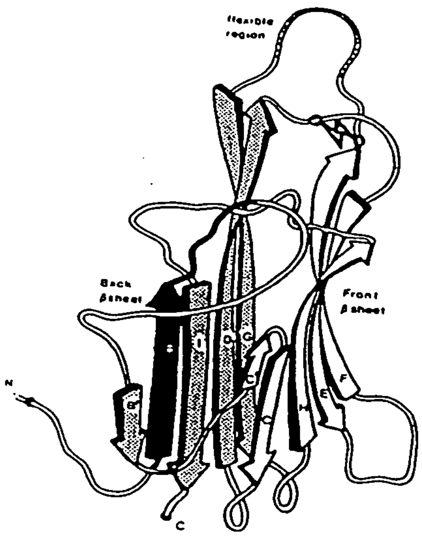


Fig. 4b

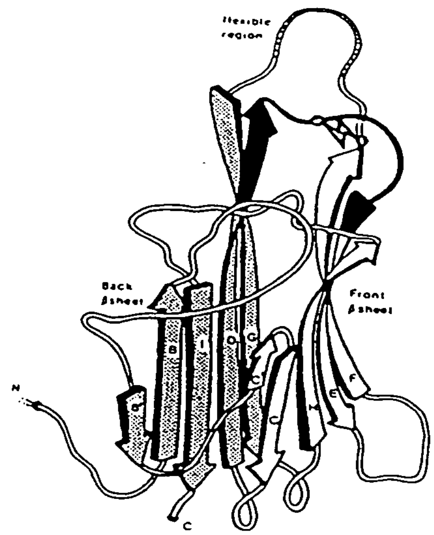
```
#     1          11         21         31         41         51         61         71
WT    VRSSSRTPSD KPVAHVVANP QAEGQLQWLN RRANALLANG VELRDNQLVV PSEGLYLIYS QVLFKGQGCP STHVLLTHTI
2-1   ---------Q YIKANSKFIG ITEL------ ---------- ---------- ---------- ---------- ----------
2-3   ---------- ---------- ---------- ---------- ---------- ---------- ----QYIKANSKFIGITEL--
2-4   ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
2-5   ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
2-7   ---------- ---------- ---------- ---------- ---------- ---------- ---------- -----QYIKA
30-1  ---------F NNFTVSFWLR VPKVSASHLE ---------- ---------- ---------- ---------- ----------
30-2  ---------- ---------- ---------- ---------F NNFTVSFWLR VPKVSASHLE ---------- ----------
30-3  ---------- ---------- ---------- ---------- ---------- ---------- ---FNNFTVS FWLRVPKVSA
30-4  ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
30-5  ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------

#     81         91         101        111        121        131        141        151
WT    SRIAVSYQTK VNLLSAIKSP CQRETPEGAE AKPWYEPIYL GGVFQLEKGD RLSAEINRPD YLDFAESGQV YFGIIAL
2-1   ---------- ---------- ---------- ---------- ---------- ---------- ---------- -------
2-3   ---------- ---------- ---------- ---------- ---------- ---------- ---------- -------
2-4   ---------- ---------- ---------- ---QYIKANS KFIGITEL-- ---------- ---------- -------
2-5   ---------- ---------- ---------- ---------- ---------- -QYIKANSKF IGITEL---- -------
2-7   NSKFIGITEL ---------- ---------- ---------- ---------- ---------- ---------- -------
30-1  ---------- ---------- ---------- ---------- ---------- ---------- ---------- -------
30-2  ---------- ---------- ---------- ---------- ---------- ---------- ---------- -------
30-3  SHLE------ ---------- ---------- ---------- ---------- ---------- ---------- -------
30-4  ---------- ---------- -----FNNFT VSFWLRVPKV SASHLE---- ---------- ---------- -------
30-5  ---------- ---------- ---------- ---------- ---------- -FNNFTVSFW LRVPKVSASH LE-----
```

Fig. 5a

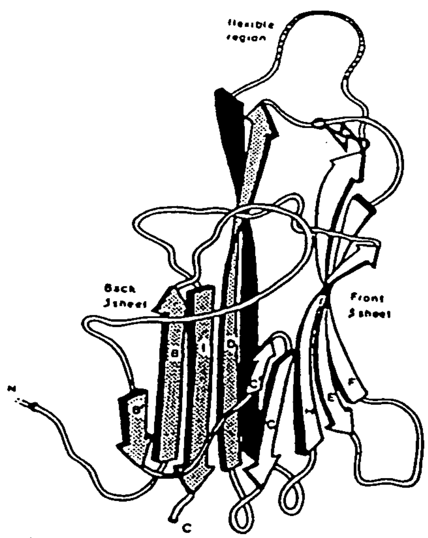
TNFα analogs with the P2 epitope inserted.



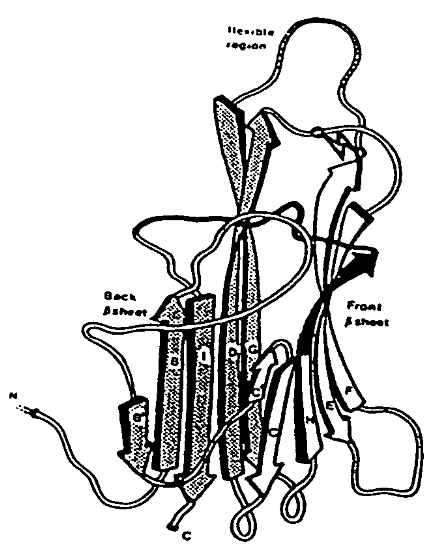
TNF2-1



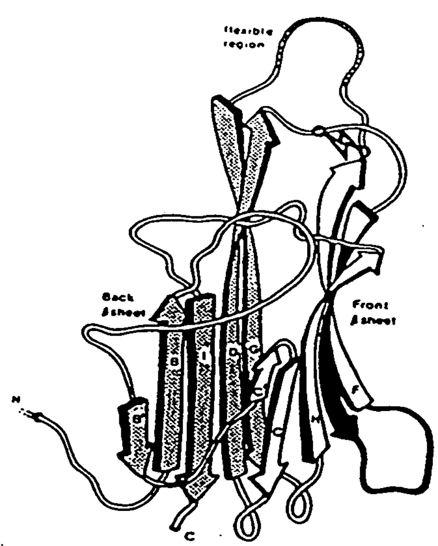
TNF2-3



TNF2-4



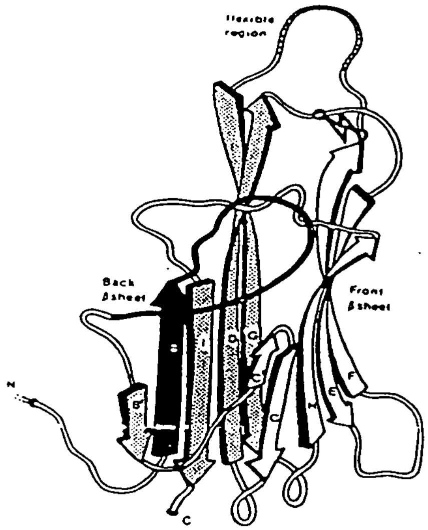
TNF2-5



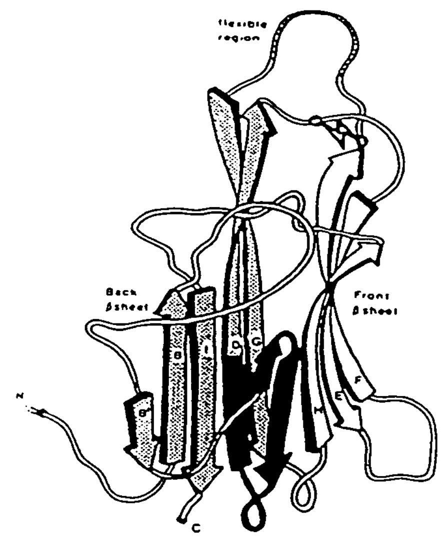
TNF2-7

Fig. 5b

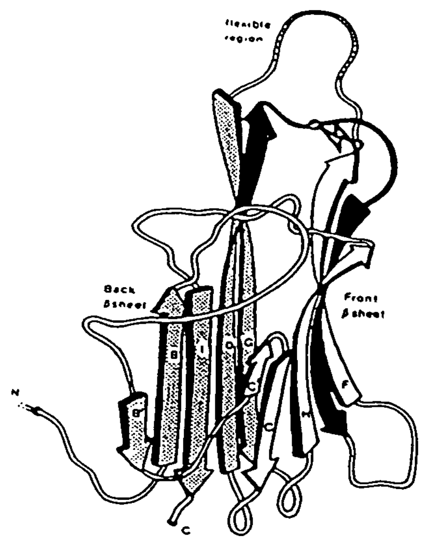
TNFα analogs with the P30 epitope inserted.



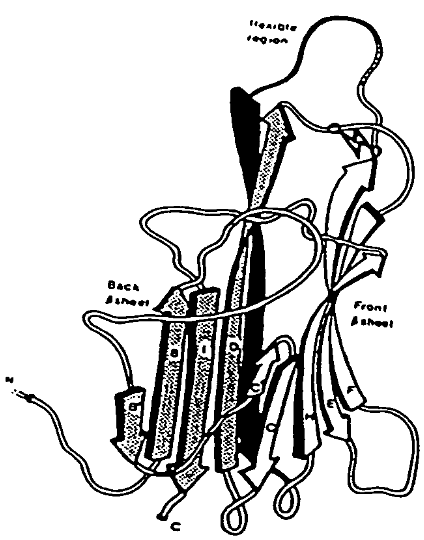
TNF30-1



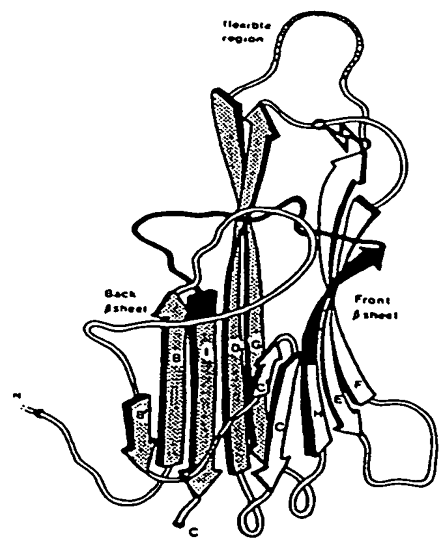
TNF30-2



TNF30-3



TNF30-4



TNF30-5

Fig. 6

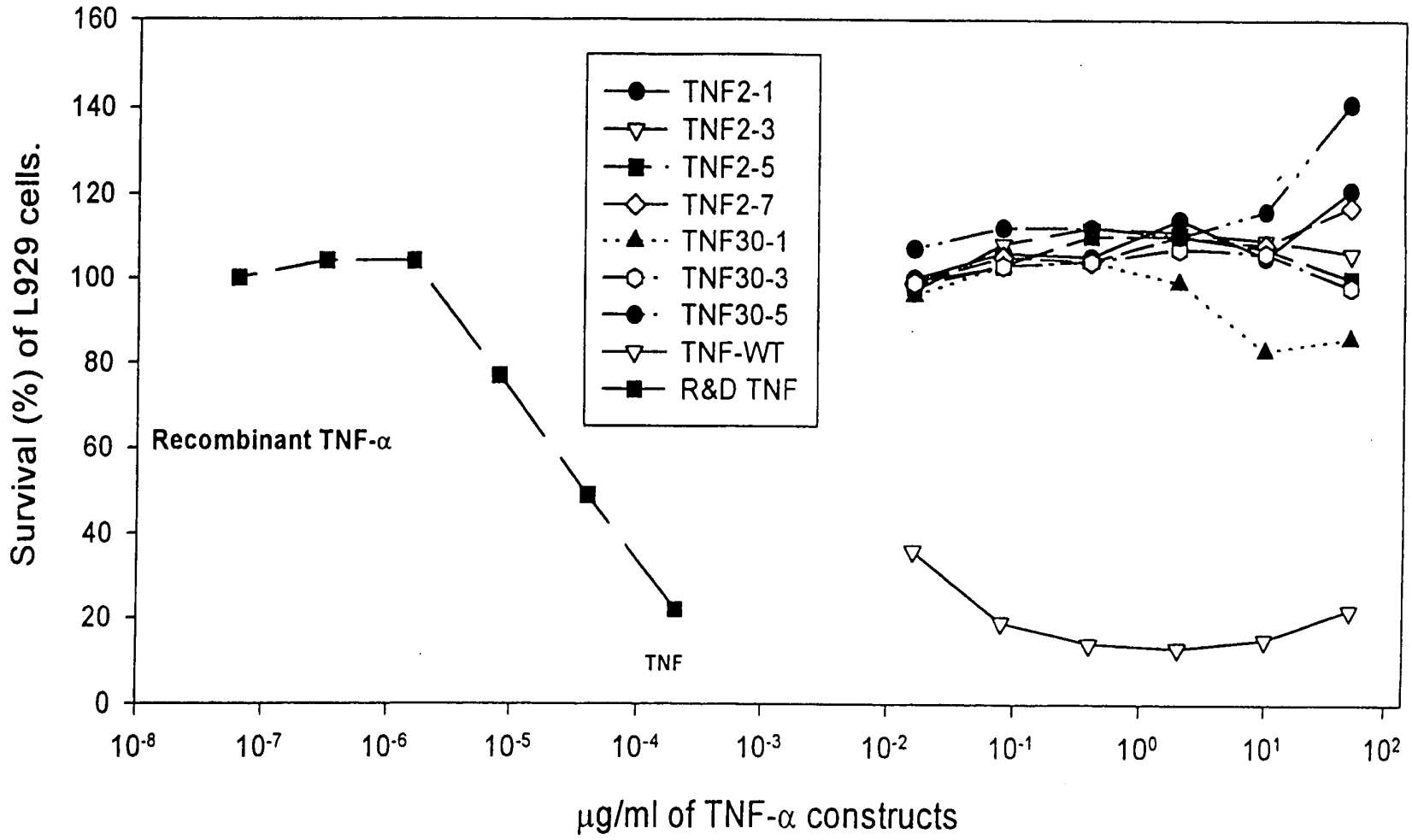


Fig. 7

The anti human TNFα antibody responce in rabbits.

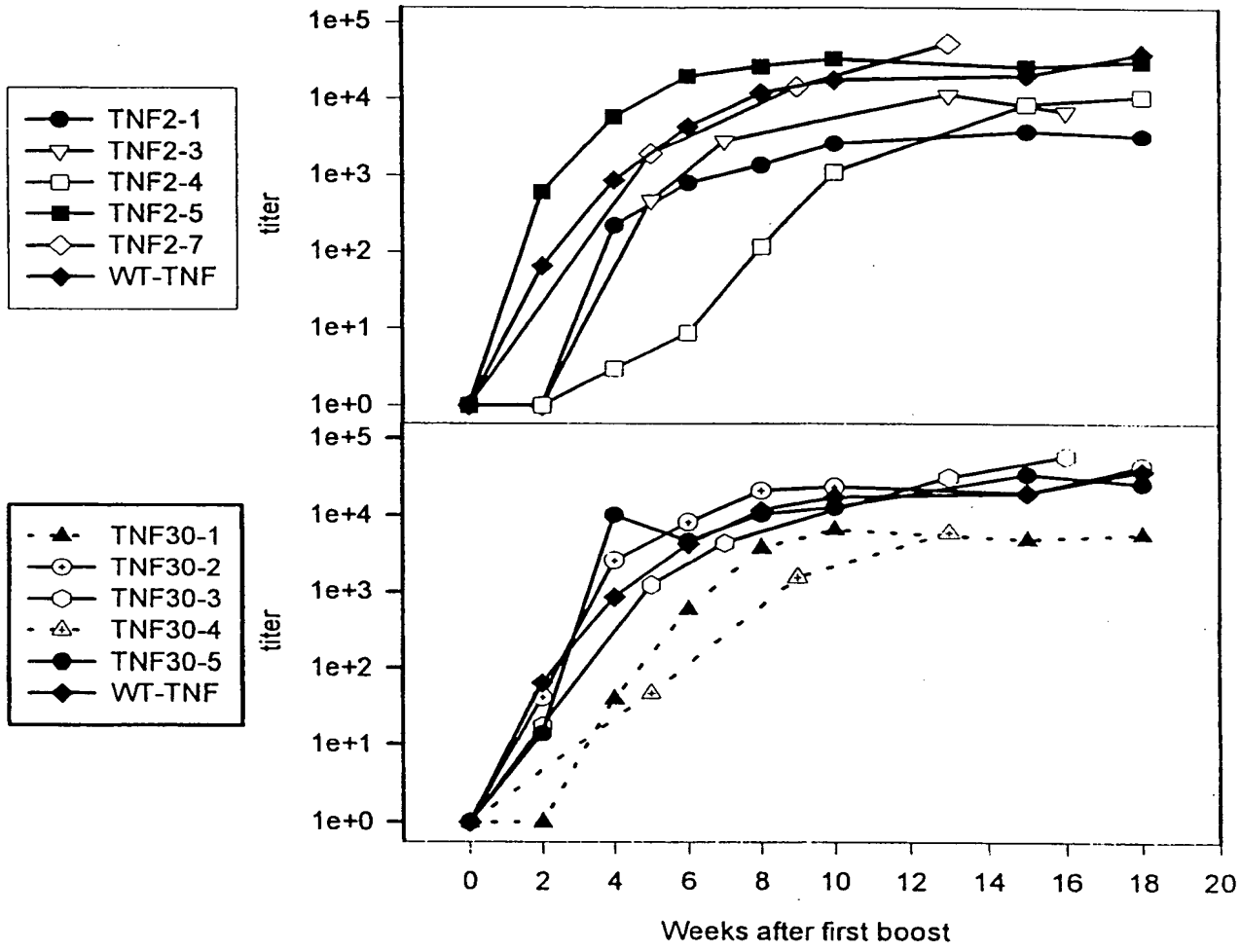


Fig. 8

The ability of P2/P30 modified human TNFα molecules to induce neutralizing antibodies as measured in the L929 cell assay.

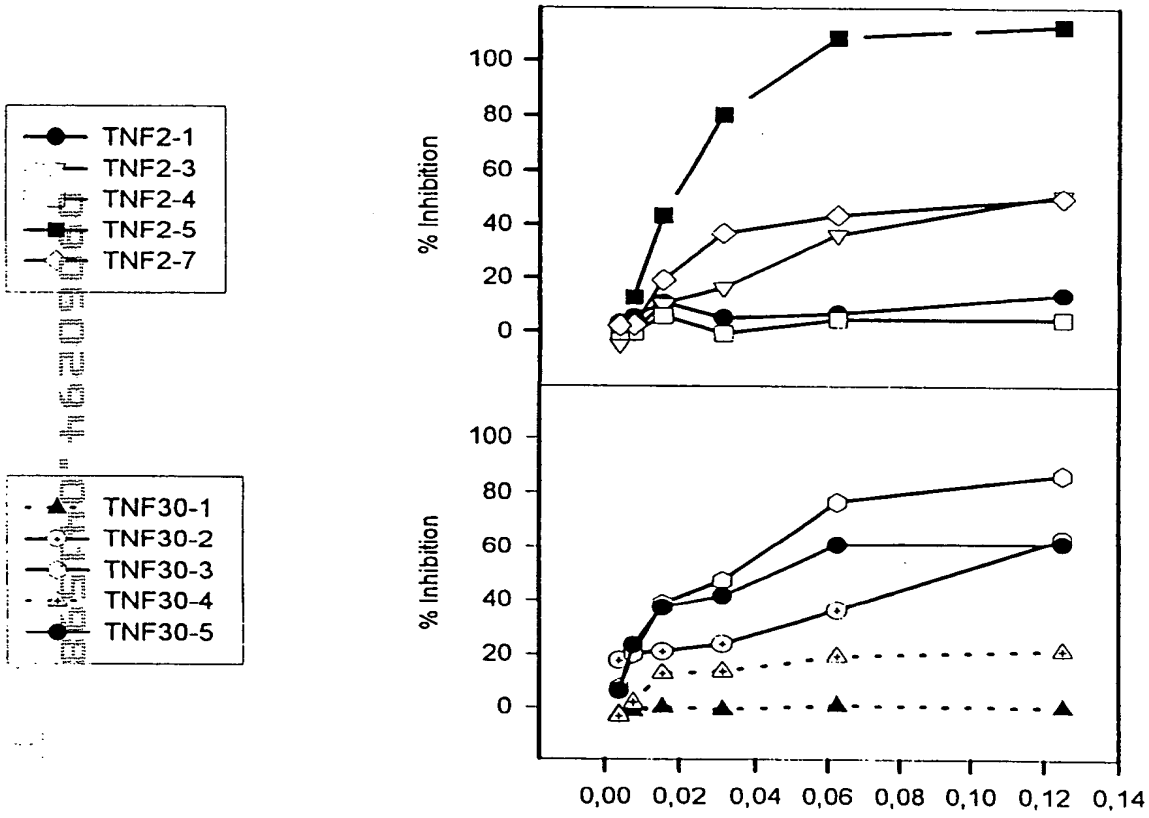
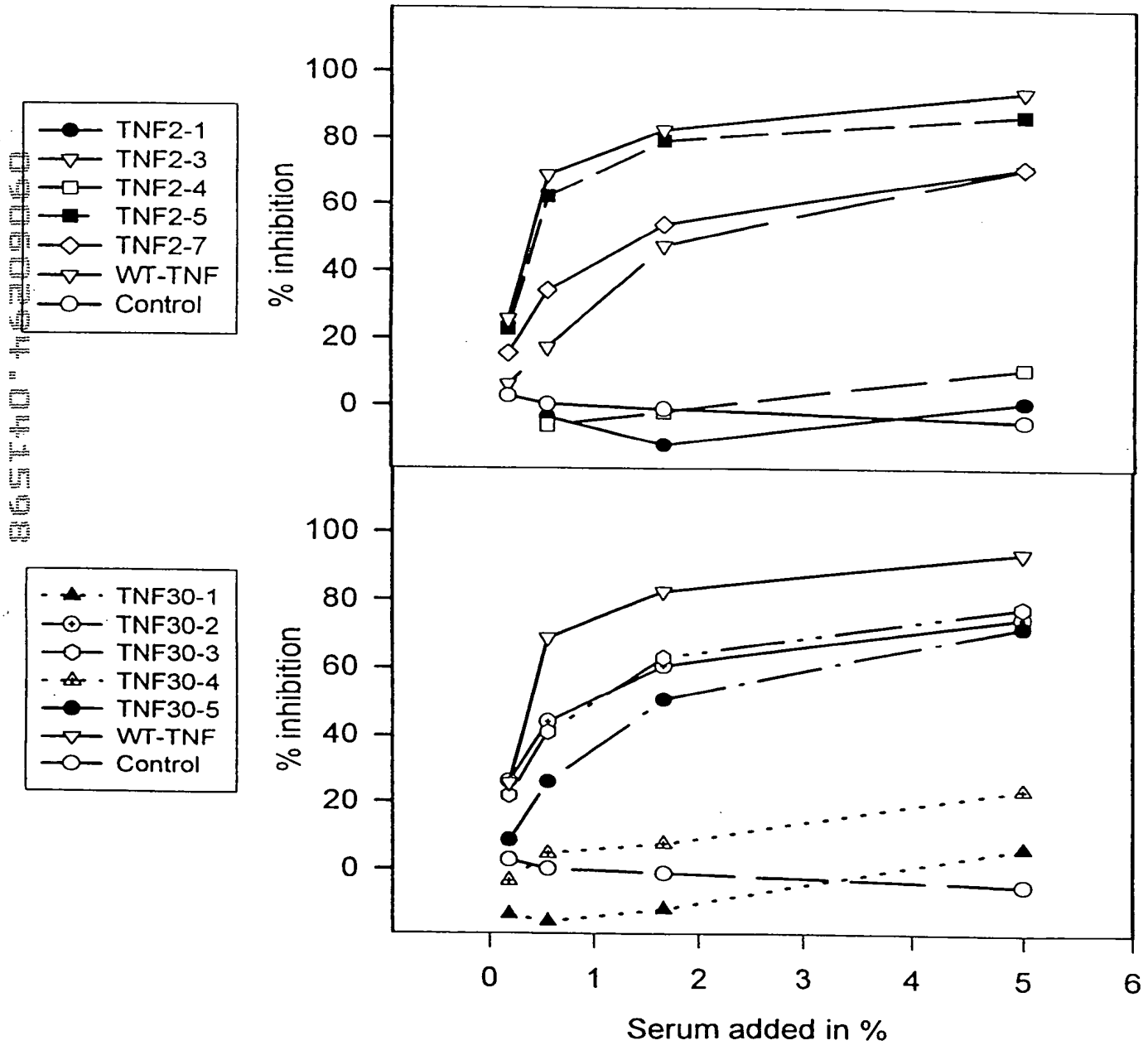


Fig. 9

The ability of P2/P30 modified human TNFαmolecules to induce neutralizing antibodies as measured in the receptor assay



ig. 10

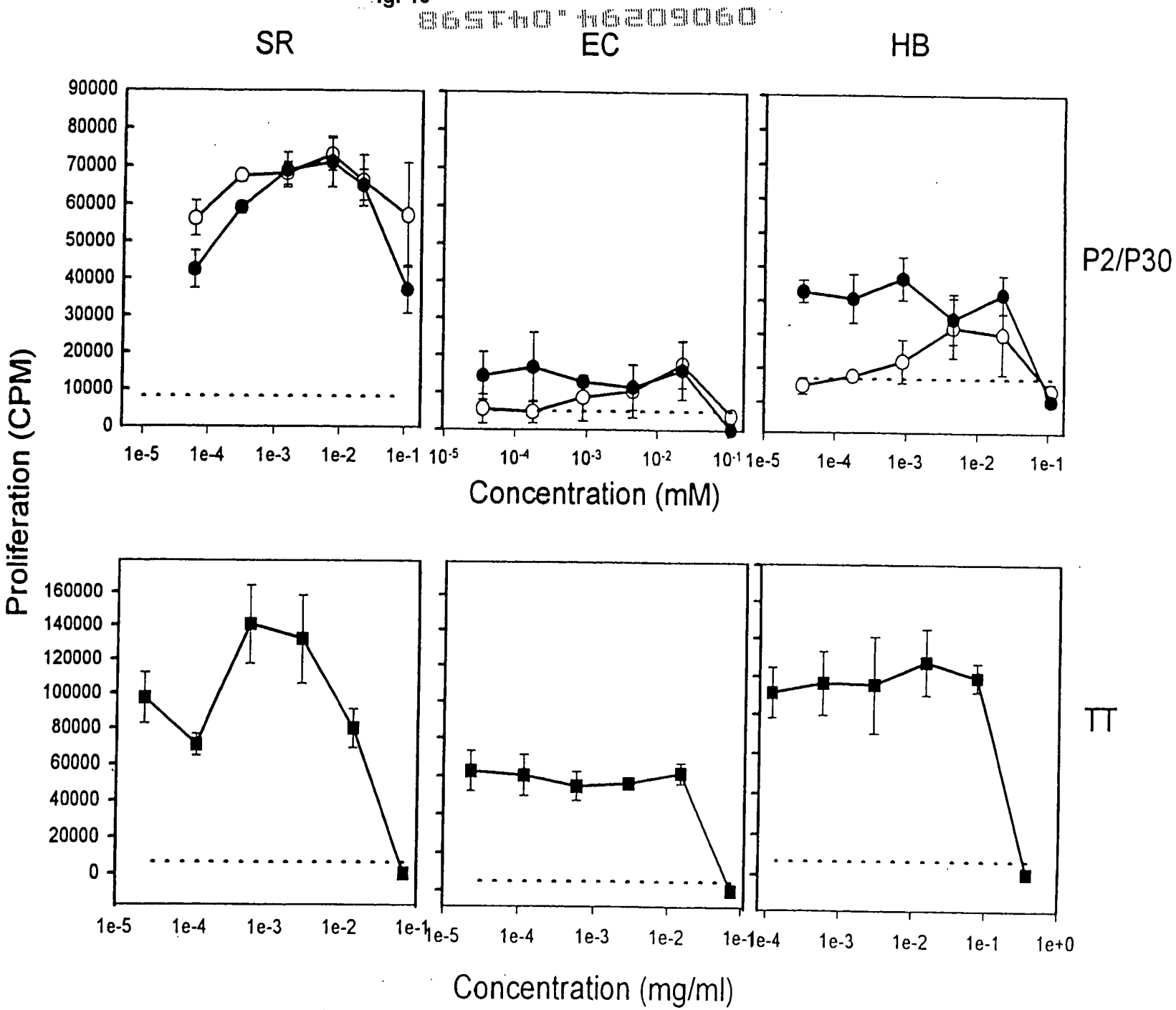

SR
EC
HB
P2/P30
TT
Proliferation (CPM)
Concentration (mM)
Concentration (mg/ml)
90000
80000
70000
60000
50000
40000
30000
20000
10000
0
160000
140000
120000
100000
1e-5
1e-4
1e-3
1e-2
1e-1
1e+0

Fig. 11

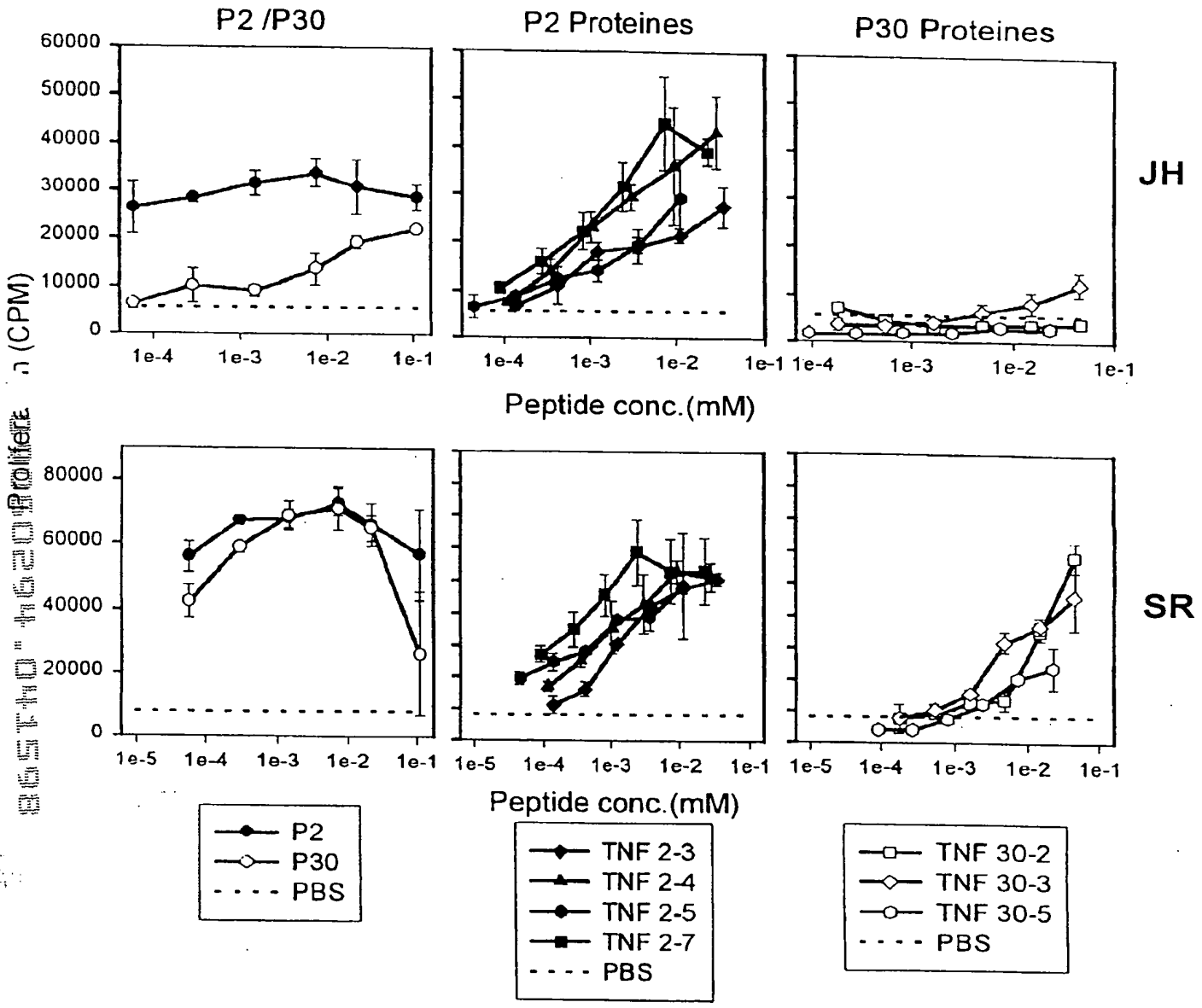

P2 /P30
P2 Proteines
P30 Proteines
JH
SR
60000
50000
40000
30000
20000
10000
0
80000
1e-5
1e-4
1e-3
1e-2
1e-1
Peptide conc.(mM)
P2
P30
PBS
TNF 2-3
TNF 2-4
TNF 2-5
TNF 2-7
PBS
TNF 30-2
TNF 30-3
TNF 30-5
PBS

Fig. 12

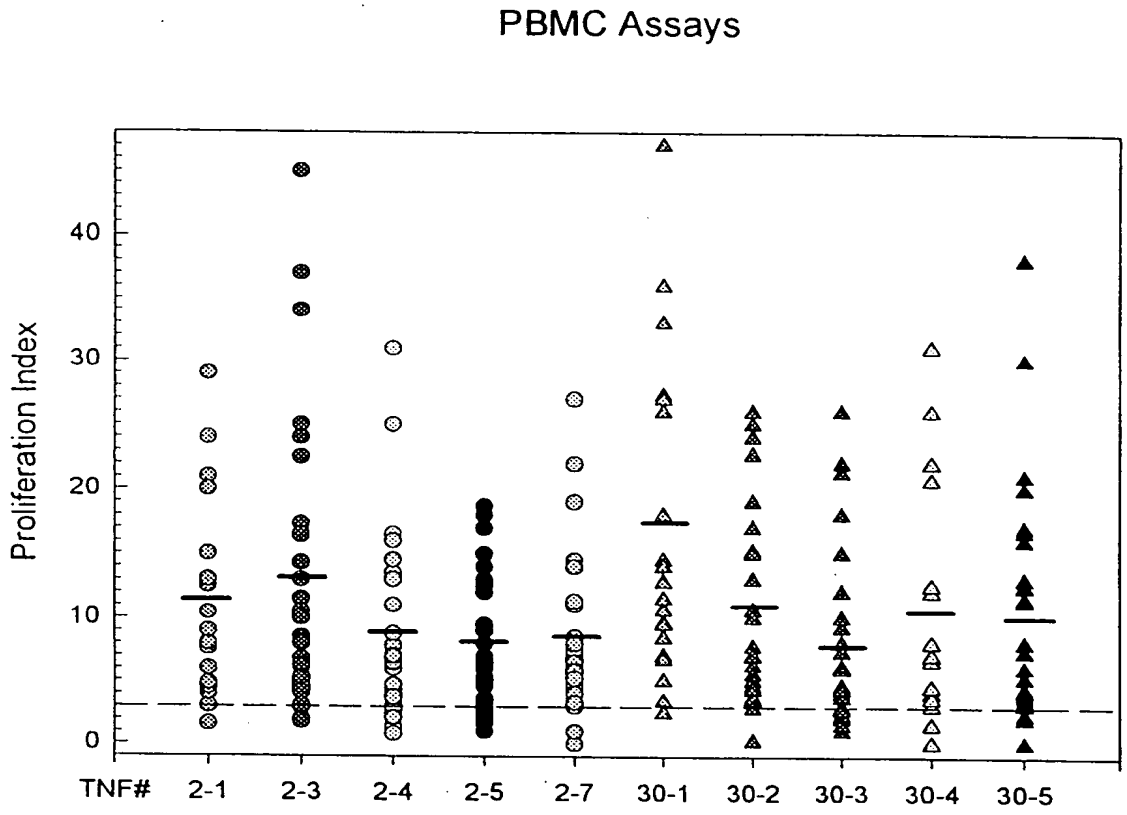

PBMC Assays
Proliferation Index
0
10
20
30
40
TNF#
2-1
2-3
2-4
2-5
2-7
30-1
30-2
30-3
30-4
30-5

Fig. 13

The PBMC responce against P2 and P30 modified TNFα proteins in P2 and P30 specific responders, respectively.

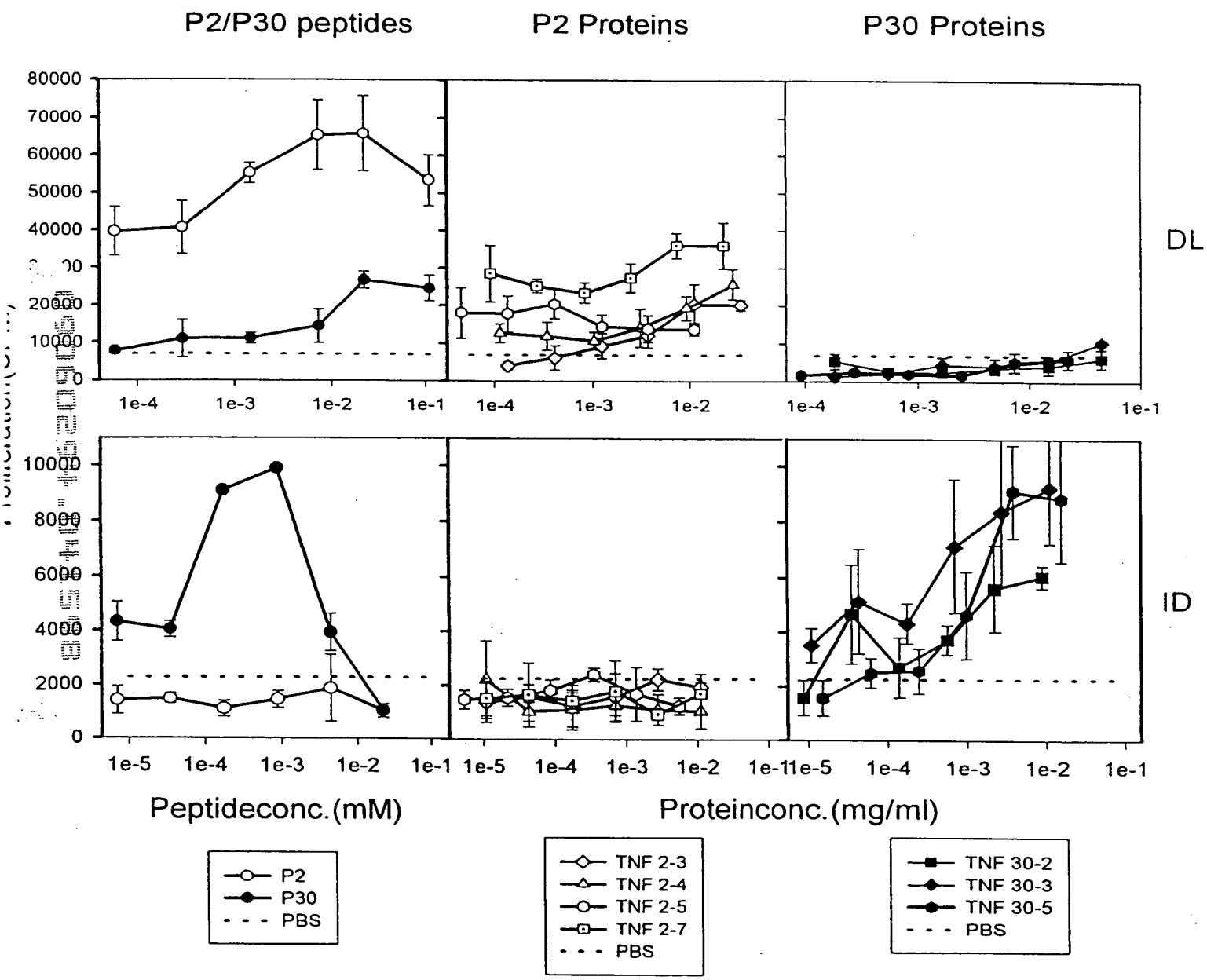


Fig. 14

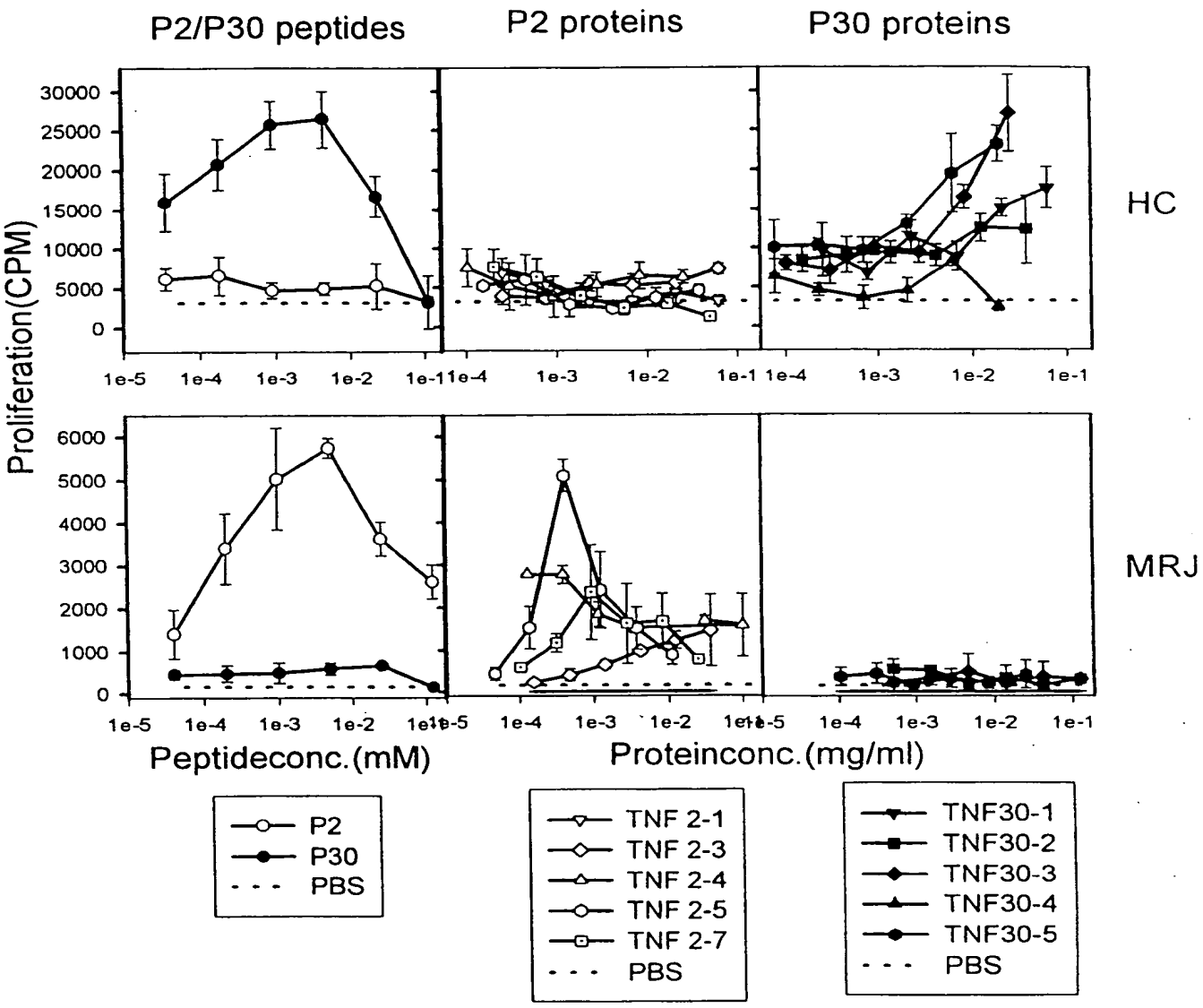

P2/P30 peptides
P2 proteins
P30 proteins
Proliferation(CPM)
HC
MRJ
Peptideconc.(mM)
Proteinconc.(mg/ml)
P2
P30
PBS
TNF 2-1
TNF 2-3
TNF 2-4
TNF 2-5
TNF 2-7
PBS
TNF30-1
TNF30-2
TNF30-3
TNF30-4
TNF30-5
PBS

Fig. 15

The influence of flanking amino acids on the T cell recognition of P2 and P30

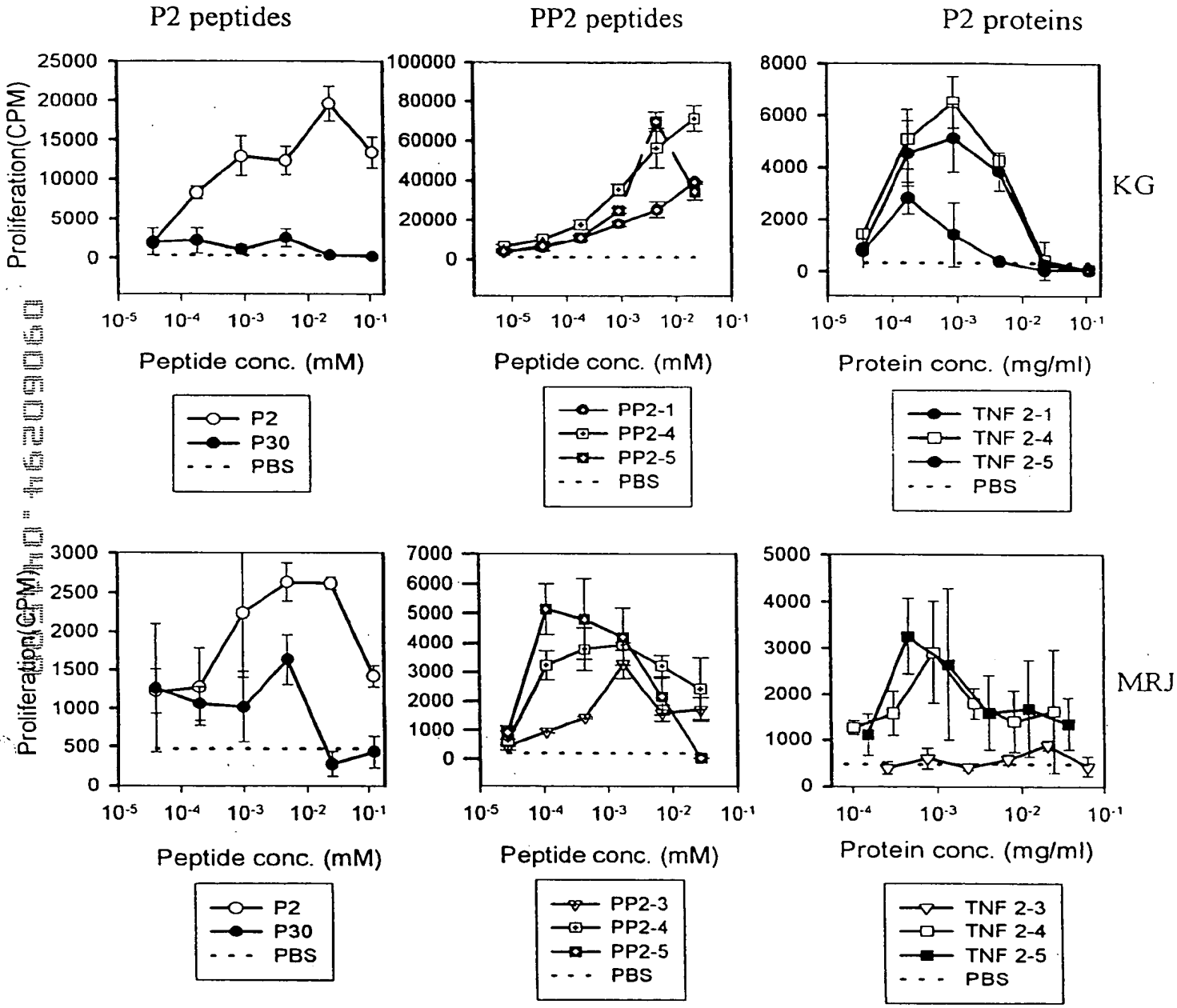


Fig. 16

Mutation Strategy